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2.	Patent application number *(The Patent Office will fill in this part)*	**9826890.7**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	**DEVGEN nv** **WOLVENDREEF 26g** **B 8500** **BELGIUM**
	Patents ADP number *(if you know it)*	7654911001
	If the applicant is a corporate body, give the country/state of its incorporation	Belgium
4.	Title of the invention	**METHOD FOR SCREENING COMPOUNDS**
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* Patents ADP number *(if you know it)*	**BOULT WADE TENNANT** **27 FURNIVAL STREET** **LONDON** **EC4A 1PQ** **42001**

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number (if you know it)	Date of filing *(day/month/year)*

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8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	**YES**

METHOD FOR SCREENING COMPOUNDS

The present invention is concerned with the field of 'genetic pharmacology'. Specifically, it relates to methods which can determine, among other things, whether a compound has potential pharmacological activity, whether a compound interacts with a particular gene or biochemical pathway in man or animals, what side effects are likely to be associated with a particular pharmaceutical compound and/or the mode or modes of action of any compound with biological activity. Additional uses for the methods of the invention include the assignment of function to particular genes or assignment of genes and their encoded proteins to particular biochemical pathways. In particular, the invention relates to the use of a nematode worm, for example Caenorhabditis elegans, and libraries of such worms in the aforementioned methods. These new methods are able to enhance and accelerate the drug discovery process.

Prior to the early 1990's the search for new compounds having the potential to combat human or animal disease was often begun by taking a compound known to have a particular pharmacological activity, synthesising structurally related variants and then testing those variants against the known target.

The test against the target might be carried out *in vivo*, for example by use of animal models of a human disease. Alternatively, if a particular molecule was known to be implicated in the progress of a disease, the compounds could be tested for interaction with the molecule *in vitro*. The limitations of such methods are that in the event of a negative result no other information about the pharmaceutical potential of the compound tested is

all. Furthermore, rather than starting from a compound of known 'activity' and relying on theoretical structure/function relationships to synthesise new candidate compounds, vast libraries of compounds, of uniform activity can be very rapidly synthesized in an automated manner by combinatorial chemistry. Thus, there is now potential to screen thousands of compounds against thousands of genes and the proteins they encode in very rapid high throughput screens (HTS) and to link compounds to genes and genes to disease.

The present inventors have discovered that these new technologies for drug discovery can conveniently be married with a particular multicellular organism, a nematode worm, *C. elegans*, which has been well characterised genetically and morphologically. They have thereby developed new methods, which are extremely powerful, rapid and convenient and can play an essential part in a drug discovery program.

C. elegans is a nematode worm which occurs naturally in the soil but can be easily grown in the laboratory on nutrient agar inoculated with bacteria, preferably *E. coli*, on which it feeds. Each worm grows from an embryo to an adult worm of about 1 mm long in three days or so. As it is fully transparent at all stages of its life, cell divisions, migrations and differentiation can be seen in live animals. Furthermore, although its anatomy is simple its somatic cells represent most major differentiated tissue types including muscles, neurons, intestine and epidermis. Accordingly, differences in phenotype which represent a departure from that of a wild-type worm are relatively easily observed, either directly by microscopy or by using selective staining procedures. Many *C.* elegans mutants have been

characteristic such as, for example, pharyngeal pumping rate or defecation frequency. Since that single characteristic may be determined by expression of a number of genes and the operation of several biochemical pathways such a crude assessment of phenotype is not sufficient to establish a link between any one gene or pathway and a compound to which the worm has been exposed. As such the procedure would not be sensitive enough for resolution of the properties of thousands of compounds in a high throughout compound screen. An additional problem with the proposals of the prior art is that known phenotypic characteristics have all been described differently by different workers in the *C. elegans* field. Phenotype descriptions in the literature largely omit aspects not directly related to or not recognised to be related to the principle interest of the individual researcher. There is no standard nomenclature to identify a specific change. Without this it is impossible to equate newly observed phenotypes with particular known phenotypes for comparison purposes.

The present inventors have developed methods which solve these problems and thereby have converted *C. elegans* into a really useful tool in the drug discovery field. Specifically, in respect of each worm a 'phenotype profile' or 'fingerprint' is established based on looking for plurality of changed characteristics in a particular mutant or worm which has been exposed to an environmental change or a compound. Furthermore, each profile is scored by following a strict standard protocol of measurement and a standard description is applied to each characteristic. The determination of a phenotypic profile in this way for a plurality of mutants or

different defect, and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

Caenorhabditis *elegans* is the preferred nematode worm although the method could be carried out with other nematodes and in particular with other nematodes of the Caenorhabditis genus.

It is preferred to establish the phenotypic profile on the basis of the observation and scoring of at least three different characteristics, preferably at least six characteristics and more preferably at least ten characteristics. It will be appreciated that the more differences which can be scored between a worm with a genetic defect and a worm without the defect the better the resolution between different mutants. Although not limited to such, at least one of the plurality of changed characteristics which can be looked for and scored may be selected from the list shown in Table 1, and possibly each of all the changed characteristics scored is one of those shown in Table 1. For comparison purposes it is essential that the scored characteristics are represented in the same order for each profile. For standardization of procedure between different workers or to facilitate automation, observation and scoring of the characteristics could be carried out in a pre-determined order according to a standard protocol. However, this is not essential to the operation of the method. In its simplest form and as shown in Example 5, the characteristics are recorded in a binary manner as 'present' or 'not present' based on deviations from wild-type worms.

It is desirable to establish a library which

target. A list of human diseases for which a particular gene has been implicated is given in the paper by J. Ahringer (see above) and also provided by OMIM. Center for Medical Genetics, John Hopkins University and National Biotechnology Information, National Library of Medicine, 1996. http//www.ncbi.nlm.nih.gov/omim/, although these lists are not necessarily exhaustive.

It is easy to establish transgenic lines in *C. elegans* and the methodology is described in Craig Mello and Andrew Fire, Methods in Cell Biology, Vol 48 Ed. H.F. Epsein and D.C. Shakes, Academic Press, pages 452-480.

A form of the worm which may show a change in phenotype and may therefore be subject to profiling as described above is one in which the genetic defect and/or transgene and/or reporter gene is only present in a sub-set of the cells of the worm. It is possible for just the cells of a particular tissue to be the subject of a genetic manipulation.

The worm which is to be subject to determination of its phenotypic profile can be cultured by methods well-known in the art. *C. elegans* can grow on nutrient agar which has first been inoculated with bacteria on which the worms feed. Suitable culture methods are described in Rand and Johnson (see above) and in the examples given herein. Observation of any changed characteristics which will determine the profile may be carried out using light microscopy, differential interference contrast optics or fluorescence microscopy. In addition immuno-chemical detection, colorimetric detection, or detection of fluorescence, luminescence or radioactive labels may be used. In some cases the changed characteristics may be biochemical only and might be detected, for

(logical OR) the profiles of all the mutations, whether they have been generated at the same time or not. It is possible, however, to handle the mutations separately and make more detailed connections, for example, concerning protein domains in case the similarity of phenotypes cluster with the sites of the mutations.

Described above are methods for constructing a library of phenotypic profiles for worms with a plurality of genetic defects or a library of mutant worms. However, in accordance with a second aspect the present invention provides a method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to a compound,

(b) observing any changes in identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile associated with said compound,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different compounds, and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

Methods for culturing *C. elegans* in the presence of a test compound are described by Rand and Johnson

or banks of worms whose phenotypic profile has been altered by exposure to compounds.

In particular embodiments assays may be carried out with several concentrations of the same compound, and/or with mixtures of compounds. For example compounds from compound libraries may each be tested individually or with one or more other influencing compounds. Furthermore, such compound testing protocols may be executed against identical worms or multiple mutant and/or transgenic backgrounds. In a particular example a panel of worm strains, covering a wide range of biochemical pathways and cellular activities by means of mutations in particular pathways, as well as reporter genes, is used for testing compounds. For each compound, potentially at several concentrations, a profile is recorded for the observable phenotypes of each of the worm strains, either in parallel or sequentially.

In a third of its aspects the invention provides a method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to an environmental change,

(b) observing any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said change,

(d) simultaneously or sequentially repeating

mutants may indicate the likely gene or biochemical pathway with which the compound interacts in the worm. Other databases can then be searched for a match of the worm gene with an equivalent human gene. The human gene might already be associated with a human disease as could be determined for example, from the OMIM database mentioned above. Thus, by use of the worm screen a potential candidate drug can be identified. The discovery of the mode of action of a compound with known pharmacological or biochemical activity is facilitated by comparing its phenotypic profile in the worm with the mutant library or environmental change library of profiles to identify possible targets for the compound. Other possibilities include finding a new potential medical indication of a known compound, a medical indication for a novel compound, an alternative method of treatment of a known disease or an indication of the reason for the side effect exhibited by some known pharmaceuticals. Testing worms with compounds, scoring the phenotypic profile in the novel manner described herein and then searching previously established libraries of profiles can potentially achieve all those goals. Once a compound has been identified as having the potential to be a therapeutic agent it can be processed through the more traditional drug discovery routes. The compound can be tested in more specific *in vitro* tests based on the new knowledge of the target for the compound and in animal models of the target disease. Structural variants then can be generated by medicinal chemistry with a view to improving activity.

The invention will now be described with reference to the accompanying examples.

TABLE 1.

ORG	ID	PROFILED BY	DATE

1. Compound specific phenotypes

Phenctype									Comment
1.1. Disappeared									
1.2. Determining compound action									
1.2.1 acute death without tracks									
1.2.2 acute death with tracks									
1.2.3 burst									
1.2.4 dissolving									
1.2.5 pale									
1.3. Compound response									
1.3.1 tracks not in center									
1.3.2 tracks inside									
1.3.3 tracks more outside									
1.3.4 tracks only outside									
1.3.5 tracks invisible									
1.3.6 attraction									
1.3.7 avoidance (try to avoid)									
1.3.8 avoidance (try to escape)									
1.4. Course of compound response									
1.4.1 immediate response									
1.4.2 delayed response									
1.4.3 progression of phenotype									
1.4.4 shift of phenotype									
1.4.5 recovered from exposure									
1.4.5.1 compound inactive									
1.4.5.2 irreversible									
1.4.5.3 adapted to compound									
1.5. Later exposed worm different									
1.5.1 weaker									
1.5.2 worse									
1.5.3 lower penetrance									
1.5.4 higher penetrance									
1.5.5 not affected									

2. Viability

Phenotype									Comment
abnormal									
2.1. Dead Adult (P0; during 3days)									
2.2. Partial lethality									
2.2.1 Few dead eggs									
2.2.2 Few dead larvae									
2.3. Embryonic arrest of F1									
2.3.1 Leakyness									
2.3.2 Appearance of eggs									
2.3.2.1 dark eggs									
2.3.2.2 bright eggs									
2.3.2.3 two-fold or older									
2.3.2.4 irregular egg-size									
2.4. Larval arrest of F1									
2.4.1 Leakyness									
2.4.2 at L1									
2.4.3 at L2									
2.4.4 at L3									
2.4.5 at L4									
2.5. Embryonic arrest of F2									

TABLE 1. (CONTINUED)

4.2.3.1 *dystrophy ventral side*									
4.2.3.2 *dystrophy dorsal side*									
4.2.3.3 *dystrophy left side*									
4.2.3.4 *dystrophy right side*									
4.2.4 only head bent									
4.2.5 hammer head									
4.2.6 swollen									
4.2.7 rounded									
4.2.8 short and rounded									
4.2.9 tapering									
4.2.10 notched									
4.2.11 vacuoles only in head									
4.2.12 autodecapitation									
4.3. Body defects									
4.3.1 bent body									
4.3.2 U-shaped									
4.3.3 humpback (dorsal lumps)									
4.3.4 truncated									
4.3.5 withered									
4.3.6 twisted									
4.3.7 spindle-shaped									
4.3.8 scrawny									
4.3.9 fat									
4.3.10 pale									
4.3.11 pale with dark spots									
4.3.12 clear									
4.3.13 extensions, protrusions									
4.3.14 fluid-filled									
4.3.15 full of vacuoles									
4.4. Tail defects									
4.4.1 only tail truncated									
4.4.2 knob-like									
4.4.3 tapering									
4.4.4 only tail withered									
4.5. Cuticle defects									
4.5.1 blistered									
4.5.1.1 *symmetrically*									
4.5.1.2 *around the head*									
4.5.1.3 *around the pharynx*									
4.5.1.4 *around the body*									
4.5.1.5 *around the tail*									
4.5.2 moulting defective									
4.5.2.1 *incomplete molts*									
4.5.2.2 *supernumerary molts*									
4.5.3 burst									
4.6. Poured out									

TABLE 1. (CONTINUED)

6. Mechanotransduction (Touch with a wire and with eyelash)

Phenotype									Comment
6.1. Harsh touch response abnormal									
6.1.1 no plate drop response									
6.1.2 no movement									
6.1.3 irregular movement									
6.1.3.1 moves not forward									
6.1.3.2 moves forward abnormal									
6.1.3.3 moves not backward									
6.1.3.4 moves backward abnormal									
6.1.3.5 moves better forward									
6.1.3.6 moves better backward									
6.1.4 cramped before movement									
schrinker before movement									
6.2. Harsh touch reflex abnormal									
6.2.1 no plate drop reflex									
6.2.2 movement after prodding									
6.2.2.1 sleecy									
6.2.3 no reflex									
6.2.4 irregular reflex									
6.2.4.1 no move back reflex									
6.2.4.2 weak move back reflex									
6.2.4.3 no move forward reflex									
6.2.4.4 weak move forward reflex									
6.2.5 cramped									
6.2.6 schrinker									
6.3. Nose touch avoidance abnormal									
6.3.1									
6.4. Foraging behavior abnormal									
6.4.1									
6.5. Body touch response abnormal									
6.5.1									

7. Sensory system

Phenotype									Comment
abnormal									
7.1. Avoidance of bacteria									
7.2. Bordering behavior									
7.3. Chemotaxis defective									
7.3.1 attraction									
7.3.2 avoidance									
7.4. Thermotaxis defective									
7.4.1 attraction									
7.4.2 avoidance									

8. Environmental response

Phenotype									Comment
abnormal									
8.1. Osmolarity sensitive									
8.2. Thermotolerance changed									
8.3. UV Resistance changed									
8.4. Oxygen sensitiv									

TABLE 1. (CONTINUED)

13. Vulva

Phenotype									Comment
abnormal									
13.1. Morphology defects									
13.1.1 defective vulva									
13.1.2 protruding vulva									
13.1.3 multi vulva (number)									
13.1.4 no vulva									
13.1.5 leaky vulva									
13.1.6									
13.1.7									

14. Fertility

Phenotype									Comment
abnormal									
14.1. Brood size abnormal									
14.1.1 smaller									
14.1.2 larger									
14.2. Egg laying defect									
14.2.1 no egg retention									
14.2.2 immediate Egl									
14.2.3 progressive Egl									
14.2.4 egg laying defective									
14.2.4.1 weak Egl									
14.2.4.2 strong Egl									
14.2.5 bloated worms									
14.2.5.1 weak bloating									
14.2.5.2 strong bloating									
14.2.5.3 bags of worms									
14.2.6 no egg laying									
14.3. Only oocytes									
14.4. Steril									
14.5. Maternal-effect steril									

15. Male

Phenotype									Comment
abnormal									
15.1. Frequency									
15.1.1 high incidence of males									
15.2. Mating defective									
15.3. Morphology									
15.3.1 leptoderan tail									
15.3.2 scrawny									
15.3.3 copulatory plug									
15.4. Mating behaviour									
15.4.1 defective sensory contact									
15.4.1.1 no response to dorsal contact									
15.4.1.2 no response to ventral contact									
15.4.2 defective backing									
15.4.2.1 no backing									
15.4.2.2 no continued backing									
15.4.3 defective turning									
15.4.3.1 loose turns									
15.4.3.2 stop at the tail									
15.4.3.3 slide off the tail									
15.4.4 defective vulval localisation									
15.4.5 defective spicul insertion									

TABLE 2.

plate	well	by	date
negative control	positive control	finished	confirmed (≥ 3 worms)
no effect	unspecific effect	needs to be applied at lower concentrations	needs to be profiled

day 0

compound
invisible
coloured
droplets
crystals
complete crust

bacteria
normal lawn
grown as ring
thin
crust
died

worm
happy
run away
irregular movement
slow movement
no movement

day 1

appearance
healthy
slightly unhealthy
slightly starved
strong starved
very sick

worm gone
lost
suicide
in agar
starved outside
died in compound

replaced by
number & stage
left progeny

movement
normal
tracks more outside
tracks not in center
amplitude increased, loopy
amplitude variable
amplitude decreased
enhanced movement
slow movement
no movement
specific:

body
normal gravid adult
pumping defects
light braun meesy gonad
pale with dark spots
few eggs in gonad
pharynx stuffed
foregut filled large
hindgut constipated
protruding vulva
other:

progeny
normal
reduced broodsize
younger staged
oocytes
coagulated eggs
dead eggs
dying hatchlings
crippled larvae

day 4

food
still plenty of
already finished
finished soon
outside comp.
not eatable, died

adult viability
still fertile
laying oocytes
died
died as bag of worms
missing

growth rate
normal
reduced broodsize
younger staged

movement
normal
population more outside
population not in center
amplitude increased, loopy
amplitude variable
amplitude decreased
enhanced movement
slow movement
no movement
specific:

body
normal gravid adult
pumping defects
light braun meesy gonad
pale with dark spots
few eggs in gonad
pharynx stuffed
foregut filled large
hindgut constipated
protruding vulva
other:

brood viability
dead eggs
dead larvae
larval arrest
later scoring
day of screen
day of worm

comparison of phenotypes

progeny shows P0 phenotype
similar
worse
a few only
weaker
no effect

new worms show phenotype
similar
worse
not all
weaker
not effect

stage & age
all stages
young only
late larvae and adults
adults only
old adults

comparison to other plates | *comparison to known drugs* | *comparison to known mutants*

replaced from the large pool where worms have been exposed to the compound in the same way.
The following concentrations can be used:

conc. in 10µl drop	100 mM	30 mM	10 mM	3 mM	1 mM	0.3 mM
conc. in 4ml agar	1000 µM	300 µM	100 µM	30 µM	10 µM	3 µM

Example 4
Comparison of agar assay to drop assay

A set of compounds from the pharmacopoeia have been profiled using the general protocol. The plate drop assay was compared against standard of pouring compounds into the agar as described in literature which method is designated agar assay. In the drop assay as well as in the agar assay, the compounds were added to the worm in a variety of concentrations, and the survival of the worm was observed as well as the phenotypic profile induced by the compound. The lowest concentration of a compound, still resulting in the death of the nematode was designated minimal lethal dose. The maximal concentration of a compound that did not result in the death of the nematode was designated maximal nonlethal dose. The minimal concentration of a compound that still resulted in an observable phenotype was designated minimal effective dose. The concentrations of the compounds in the agar assay were compared to the concentrations in the drop assay. From this observation one may conclude that the newly described drop assay protocol turns out to be far more efficient for most compounds. The following table lists the calculated concentration ratio needed to get the same effect with the compound in the agar assay (in 2 ml agar) rather than the drop assay (in 4 ml agar).

Mutant worms have been profiled according to the general profile protocol. Table 4 shows a summary of the profile, also called fingerprints, of one mutation of the indicated genes. Entries are binary with empty fields indicating a phenotype (deviation from negative control, here wild-type) not found assuming that it could have been observed. Any other entry including comments or quantitative data is read as observed phenotype in this binary scheme and indicated by *.

The table lists only phenotypes that do have a positive entry, not necessarily complete, leaving pages of empty fields alongside and arranged according to a particular enquiry. The upper half consists of the hierarchical categories "dauer formation phenotypes" and "body shape phenotypes" as well as their relevant subphenotypes. The lower part consists of a set of hierarchically unrelated phenotypes subsumed under the enquiry categories, "increased activity" and "decreased activity". The complete list of characteristics is to be found in Table 1.

The point of including the lower part is to show the principle of recording all observed phenotypes, that they can be used to distinguish similar phenotypic profiles in detail and that they can be arranged in order to make comparisons. In this case it is seen that the dichotomy of long versus short body length does not correlate to the dichotomy of increased versus decreased activity.

The upper part shows 5 genes (i.e. a mutation in that gene) affecting dauer formation as well as 5 genes affecting body shape in a particular combination. A mutation in one gene, daf-4, is unique in sharing the characteristics of both phenotypic groups. The following picture illustrates the phenotypic overlap as found by comparing entries in

TABLE 4.

phenotype	*daf-1*	*daf-7*	*daf-3*	*daf-14*	*daf-4 e1364*	*sma-2 e502*	*sma-3 e491*	*sma-4 e729*	*lon-1 e185*	*lon-3 e2175*
dauer formation	•	•	•	•	•					
constitutive dauer	•	•	•	•	•					
recovery defective	•	•	•	•	•					
body shape					•	•	•	•	•	•
short					•	•	•	•		
long									•	•
thin					•	•	•	•	•	•
pale					•	•	•	•	•	
irregular egg size					•	•		•	•	•
increased activity					•		•	•	•	•
enhanced movement					•		•		•	
amplitude increased									•	
head movement enhenced							•	•	•	•
foraging behaviour increased					•			•		•
pharynx pumping enhanced							•		•	
constitutive pumping							•	•	•	
no egg retention									•	•
decreased activity						•				
lay still						•				
slow movement						•				
pharyngeal pumping reduced						•				

Example 7
Comparison of phenotypes of mutations in the acetylcholine neurotransmission

C. elegans adults and larval stages that are homozygous for the mutation cha-1, unc-17, snt-1 and cat-1 have been profiled, meaning fingerprints have been generated. All phenotypes from the phenotype list are displayed that have been observed in this experiment. The phenotypes "small", "resistance to CHA inhibitors (Ric)", slow pumping" and "slow growth" are shared. This is called phenotype activity relationship (PAR, in analogy to structure activity relationship SAR). The shared phenotypes are used to identify genes in a pathway. The unshared phenotypes are used to distinguish these genes or unravel further functions in parallel or new pathways when these phenotypes are part of another PAR. The fingerprint of cat-1 is different because this gene is involved in the dopamin pathway.

TABLE 6.

Phenotype	*cha-1* ChAT (synthesis)	*unc-17* VChAT (ACh-transporter)	*snt-1 = ric-2* Synaptotag min homolog	*cat-1* VMAT (monoamine – transporter)
Coiler	X	X		
Small	X	X	X	
Slow growth	X	X	X	
Ric	X	X	X	
Slow pumping	X	X	X	
Jerky when backing	X			
Low ChAT level	X			
Pore male turning				X
Enhanced foraging behavior				X
Enhanced foraging behavior				X
Defecation defects				
Shrinker-uncs				

In this case the ventral muscles get contradicting signals and only the dorsal muscles contract properly. The result is a coiler that has only the ventral side outwards. We explain most of the phenotypes as consequence of a mislead process, here synaptic input.

scored.

5. A method as claimed in any preceding claim wherein said worm is Caenorhabditis elegans.

6. A method as claimed in any preceding claim wherein steps (a) to (c) are carried out in respect of substantially every gene in the worm genome.

7. A method as claimed in any preceding claim which includes the step of manipulating said worm to generate said defect in said at least one gene.

8. A method as claimed in any preceding claim wherein said defect is selected from the absence of expression of said gene, the reduction in expression of said gene, the over-expression of said gene, the expression of a functionally defective protein, the expression of a truncated protein, the misexpression of a protein, the ectopic misexpression of a protein, the expression of a protein of altered stability or the alteration of gene expression as a function of time.

9. A method as claimed in claim 7 or 8 wherein said manipulation is carried out on wild-type *C. elegans* or a selected mutant thereof.

10. A method as claimed in claim 9 wherein said selected mutant harbours multiple mutations.

11. A method as claimed in claim 7 or 8 wherein said manipulation is carried out on *C. elegans* carrying a reporter gene.

20. A method as claimed in any preceding claim wherein changed characteristics in said worm carrying said defect compared to a worm that does not carry said defect are identified by a pH change or a change in electrical potential.

21. A method as claimed in any preceding claim wherein said plurality of changed characteristics are scored in a predetermined order to generate said phenotypic profile.

22. A method as claimed in any preceding claim wherein the scoring of said plurality of changed characteristics is repeated at predetermined intervals of time.

23. A method as claimed in any preceding claim wherein said phenotypic profiles are stored electronically.

24. A method as claimed in any preceding claim wherein at least one of said plurality of characteristics is selected from the list shown in Table 1.

25. A method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to a compound,

(b) observing any changes in identifiable characteristics of said worm as a result of exposure to said compound,

32. A method as claimed in any one of claims 25 to 29 wherein each of said plurality of different compounds has no known pharmacological activity or biochemical interaction.

33. A method as claimed in any one of claims 25 to 29 wherein each of said plurality of different compounds is from a combinatorial library.

34. A method as claimed in any one of claims 25 to 33 wherein said worm to which said compound is exposed is wild-type *C. elegans* or a selected mutant thereof.

35. A method as claimed in claim 34 wherein said selected mutant harbours multiple mutations.

36. A method as claimed in any one of claims 25 to 33 wherein said worm to which said compound is exposed is *C. elegans* carrying a reporter gene.

37. A method as claimed in claim 36 wherein said reporter gene is LacZ or GFP.

38. A method as claimed in any one of claims 22 to 37 wherein said worm to which said compound is exposed is transgenic *C. elegans*.

39. A method as claimed in claim 38 wherein said transgenic *C. elegans* expresses a human gene.

40. A method as claimed in claim 39 wherein said human gene is a known drug target.

41. A method as claimed in claim 39 wherein said

characteristics are scored in a predetermined order to generate said profile.

49. A method as claimed in any one of claims 25 to 48 wherein the scoring said plurality of changed characteristics is repeated at predetermined time intervals.

50. A method as claimed in any one of claims 25 to 49 wherein said scoring of changed characteristics is carried out using essentially the same scoring protocol as used in a method in accordance with any one of claims 1 to 24.

51. A method as claimed in any one of claims 25 to 50 wherein said phenotypic profiles are stored electronically.

52. A method as claimed in any preceding claim wherein at least one of said plurality of characteristics is selected from the list shown in Table 1.

53. A method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to an environmental change,

(b) observing any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a

to 56 wherein said environmental change is a change in the temperature to which the worm is exposed and in step (d) each of the plurality of environmental changes comprises a change in temperature.

60. A method as claimed in any one of claims 53 to 56 wherein said environmental change comprises exposure to radiation and in step (d) each of said plurality of environmental changes comprises a different level of radiation.

61. A method as claimed in any one of claims 53 to 56 wherein said environmental change comprises exposure to a virus and in step (d) each of said plurality of environmental changes comprises exposure to a different virus.

62. A method as claimed in any one of claims 53 to 56 wherein said environmental change comprises exposure to a bacterium and in step (d) each of said plurality of environmental changes comprises exposure to a different bacterium.

63. A method as claimed in any one of claims 53 to 53 to 62 wherein said worm is *C. elegans*.

64. A method as claimed in any one of claims 53 to 63 including a further feature as defined in any one of claims 5 to 52.

65. A method as claimed in any one of claims 53 to 64 wherein said scoring of changed characteristics is carried out using essentially the same scoring protocol as used in a method in accordance with claims 1 to 52.

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile associated with said compound or combination of compounds, and

(d) comparing said profile with a library of reference profiles said library of reference profiles being obtainable by carrying out the method of any one of claims 1 to 66.

69. A method of finding an alternative treatment for a human disease which method comprises the steps of:

(a) exposing a nematode worm to a candidate compound,

(b) observing any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by carrying out a method in accordance with claim 30.

70. A method of finding a biochemical pathway in which a compound known to have pharmacological activity acts which method comprises the steps of:

(a) exposing a nematode worm to the known

72. A method as claimed in claim 71 wherein said library of reference profiles is obtainable by carrying out a method in accordance with claims 24 or 25.

73. A method of identifying the mechanism of action of any side effects associated with a compound of known pharmaceutical activity which method comprises the steps of;

(a) exposing a nematode worm to the known compound,

(b) observing any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by carrying out a method in accordance with claim 31 and/or any of claims 1 to 24.

74. A method of attributing a particular gene to a particular biochemical pathway in *C. elegans* which method comprises the steps of:

(a) exposing a nematode worm to a compound known to operate in a particular biochemical pathway,

(b) observing any changes in the identifiable characteristics of said worm as a result of exposure

(d) comparing said profile with a library of reference phenotypic profiles, said library of references profiles being obtainable by carrying out a method in accordance with any one of claims 1 to 24.

78. A method as claimed in claim 77 wherein said nematode worm is selected from wild-type *C. elegans*, a mutant *C. elegans* comprising one or more mutations, a *C. elegans* carrying a reporter gene or a transgenic *C. elegans*.

79. A method as claimed in claim 77 wherein said defect is selected from the absence of expression of said gene, the reduction in expression of said gene, the expression of a functionally defective protein, the expression of a truncated protein, the misexpression of a protein, the ectopic misexpression of a protein, the expression of a protein of altered stability or the alteration of gene expression as a function of time.

80. A method as claimed in any one of claims 77 to 79 wherein at least three, preferably at least six and more preferably at least ten changed characteristics are scored.

81. A method as claimed in any of claims 77 to 80 which includes the features described in any one of claims 19 to 24.

82. A method of constructing a library of nematode worms which method comprises the steps of:

(a) providing a worm having a defect in at least

changed characteristics to establish a phenotypic profile associated with said compound,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different compounds, and

(e) producing a library of said worms each identifiable by their phenotypic profiles.

86. A method as claimed in claim 85 wherein said phenotypic profiles are collated into a library.

87. A method as claimed in claim 85 or 86 comprising any one of the features disclosed in any one of claims 26 to 52.

88. A method of constructing a library of nematode worms which method comprises the steps of:

(a) exposing a worm to an environmental change,

(b) observing any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said change,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different environmental

(a) exposing an nematode worm to said compound or combination of compounds,

(b observing any changes in identifiable characteristics of said worm as a result of said exposure,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile associated with said compounds or combination of compounds, and

(d) comparing said phenotypic profile with a library of reference profiles wherein said library of reference profiles is obtainable by the method of any one of claims 83, 86 or 89.

93. A method of finding an alternative treatment for a human disease which method comprises the steps of:

(a) exposing an nematode worm to a candidate compound,

(b) observing any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of referenced profiles, wherein said library of

characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by the method of any one of claims 83, 86 or 89.

96. A method of identifying the mechanism of action of any side effects associated with a compound of known pharmaceutical activity which method comprises the steps of:

(a) exposing an nematode worm to the known compound,

(b) observing any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference of profiles being obtainable by the method of any one of claims 83, 86 or 89.

97. A method of attributing a particular gene to

by wild-type worms.

101. A method as claimed in claim 100 wherein said characteristics not exhibited by wild-type worms are selected from the list shown in Table 1.

102. A method as claimed in claim 100 or 101 wherein said phenotypic profile is established for a nematode worm which is selected from a worm having one or more mutations, a worm which has been exposed to a compound or combination of compounds, a transgenic worm, a worm carrying a reporter gene or a worm which has been exposed to an environmental change.

103. A method as claimed in claim 102 wherein said transgenic worm comprises a human gene.

104. A method as claimed in claim 102 wherein said compound has known pharmacological activity.

105. A method as claimed in claim 103 wherein said compound is known to be active in a particular biochemical pathway.

106. A method as claimed in claim 102 wherein said compound or combination of compounds is from a combinatorial library of compounds.

107. A compound which has potential therapeutic activity in a mammal which has been identified in a method as claimed in any one of claims 67 to 76 or 91 to 99.

108. A library of nematode worms obtainable by a method as claimed in any one of claims 82 to 90.